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The Effects of Lipid-Lowering and Antioxidant Vitamin Therapies on Flow-Mediated Vasodilation of the Brachial Artery in Older Adults With Hypercholesterolemia

James H. Stein, MD, FACC, Cynthia M. Carlsson, MD, Kristi Papcke-Benson, RN, MSN, Susan E. Aeschlimann, RDMS, RVT, Apple Bodemer, MD, Molly Carnes, MD, Patrick E. McBride, MD, MPH

Madison, Wisconsin

OBJECTIVES The goal of this study was to determine the long-term effects of statins and antioxidant vitamins on flow-mediated vasodilation of the brachial artery in older adults with hypercholesterolemia.

BACKGROUND Lipid-lowering therapy and antioxidant vitamins improve endothelium-dependent vasodilation in young and middle-aged adults with hypercholesterolemia, but their effects in older adults are not known.

METHODS Two double-blind, placebo-controlled studies were performed in individuals ≥70 years old with low-density lipoprotein cholesterol (LDL-C) ≥140 mg/dl. In the first study, 37 subjects were randomized to receive (group 1) pravastatin for six months then pravastatin and vitamin E for six additional months or (group 2) vitamin E for six months, then pravastatin and vitamin E for six additional months. In the second study, additional 17 subjects sequentially received simvastatin for six months, then simvastatin and vitamins C and E for six additional months. Flow-mediated vasodilation of the brachial artery was measured by high-resolution ultrasound.

RESULTS At baseline, subjects in both studies were similar in age (mean ± SD, 75.8 ± 4.2 years), gender, systolic blood pressure, total cholesterol (261.6 ± 37.4 mg/dl), LDL-C (180.3 ± 28.1 mg/dl), high-density lipoprotein cholesterol and triglycerides levels. Flow-mediated vasodilation was severely impaired (2.2 ± 3.9%). Both statins reduced total and LDL-C levels ($p < 0.001$); however, neither statin, antioxidant vitamin regimen nor the combination of statins and antioxidant vitamins improved flow-mediated vasodilation of the brachial artery. At baseline, nitroglycerin-mediated vasodilation also was impaired (10.7 ± 5.6%) and did not change in either study.

CONCLUSIONS Older adults with hypercholesterolemia have impaired flow-mediated vasodilation of the brachial artery that does not improve after one year of therapy with statins and antioxidant vitamins, despite significant lipid-lowering. (J Am Coll Cardiol 2001;38:1806–13) © 2001 by the American College of Cardiology

Coronary heart disease (CHD) is the leading cause of death in older men and women (1). Endothelial dysfunction contributes to the initiation, perpetuation and clinical manifestations of CHD (2–4). Normally, increased blood flow raises shear stress on endothelial cells, leading to nitric oxide-dependent arterial dilation; however, in patients with atherosclerosis or CHD risk factors, endothelial dysfunction leads to impaired flow-mediated vasodilation (FMD) or paradoxical vasoconstriction in response to increased blood flow (3–5). Flow-mediated vasodilation of the brachial and coronary arteries are correlated strongly and predict future adverse cardiovascular events (3,6–9).

Aging is associated with increased endothelial production of oxygen-derived free radicals that inactivate nitric oxide leading to impaired endothelium-dependent vasodilation, independent of coronary risk factors (10–15). Although lipid-lowering therapy and use of antioxidant vitamins may improve endothelial function in young and middle-aged adults (16–22), most studies investigating their effects had a short duration, did not control for multiple comparisons and did not include patients ≥70 years old. Furthermore, several studies have not demonstrated an improvement in endothelial function with these interventions, even in younger patients (23–28), so the long-term effects of lipid-lowering therapy and antioxidant vitamins on endothelial function in older adults are not known.

Two double-blind, placebo-controlled studies evaluated the long-term effects of lipid-lowering and antioxidant vitamin therapies in older adults with hypercholesterolemia. The first study (Pravastatin and Vitamin E in Seniors study [PAVES]) used pravastatin and vitamin E, alone and in combination. The second study (Simvastatin, Ascorbate and Vitamin E in Seniors study [SAVES]) used simvastatin and vitamins C and E. This study provides complimentary and

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Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
CHD	=	coronary heart disease
FMD	=	flow-mediated vasodilation
HDL-C	=	high-density lipoprotein cholesterol
LDL-C	=	low-density lipoprotein cholesterol
NTG	=	nitroglycerin
NTGMD	=	nitroglycerin-mediated vasodilation
PAVES	=	Pravastatin and Vitamin E in Seniors study
SAVES	=	Simvastatin, Ascorbate and Vitamin E in Seniors study

incremental information. The results of these studies are presented separately and in a combined analysis.

METHODS

Subjects. The Institutional Review Board of the University of Wisconsin Medical School approved both studies. All subjects provided informed consent before participation. Adults ≥70 years old with fasting low-density lipoprotein cholesterol (LDL-C) levels ≥140 mg/dl and triglycerides levels ≤350 mg/dl were recruited using mailings to physicians, television promotions and newspaper advertisements. Excluded were current users of tobaccos, cyclosporine or warfarin and subjects with elevated serum aspartate aminotransferase, alanine aminotransferase or creatine kinase levels >3 times the upper limit of normal. Subjects were not allowed to take cholesterol-lowering medications or vitamin supplements for four weeks before enrollment or during the studies, except as prescribed by the research protocol. Coronary heart disease was defined as a history of myocardial infarction, percutaneous or surgical coronary artery revascularization or current angina. Hypertension was defined as a systolic blood pressure ≥140 mm Hg or current use of antihypertensive medication. Diabetes mellitus was defined as a history of diabetes mellitus or use of an antiglycemic medication.

Study designs. In both studies, subjects were counseled regarding a National Cholesterol Education Program step 1 diet and were given statin-placebo capsules on enrollment (Fig. 1). After eight weeks of placebo run-in, fasting serum lipids were remeasured. Adherence was measured by pill counts, and a minimum compliance rate of 80% was required for continued participation. If the LDL-C remained ≥140 mg/dl and the subject was adherent, they were given active therapy.

Study 1—PAVES. This was a double-blind, randomized, placebo-controlled, crossover study (Fig. 1). After the pravastatin-placebo run-in, subjects were randomized to receive (group 1) pravastatin, 20 mg, each night for 26 weeks followed by pravastatin, 20 mg, and vitamin E, 400 IU (all d-alpha-tocopheryl), each night for an additional 26 weeks. Subjects in group 2 received vitamin E, 400 IU, and pravastatin-placebo nightly for 26 weeks, followed by vitamin E, 400 IU, and pravastatin, 20 mg, nightly for an additional 26 weeks.

Study 2—SAVES. This was a double-blind, placebo-controlled, sequential treatment study (Fig. 1). After the simvastatin-placebo run-in, subjects received simvastatin, 20 mg, each night for 26 weeks followed by simvastatin,

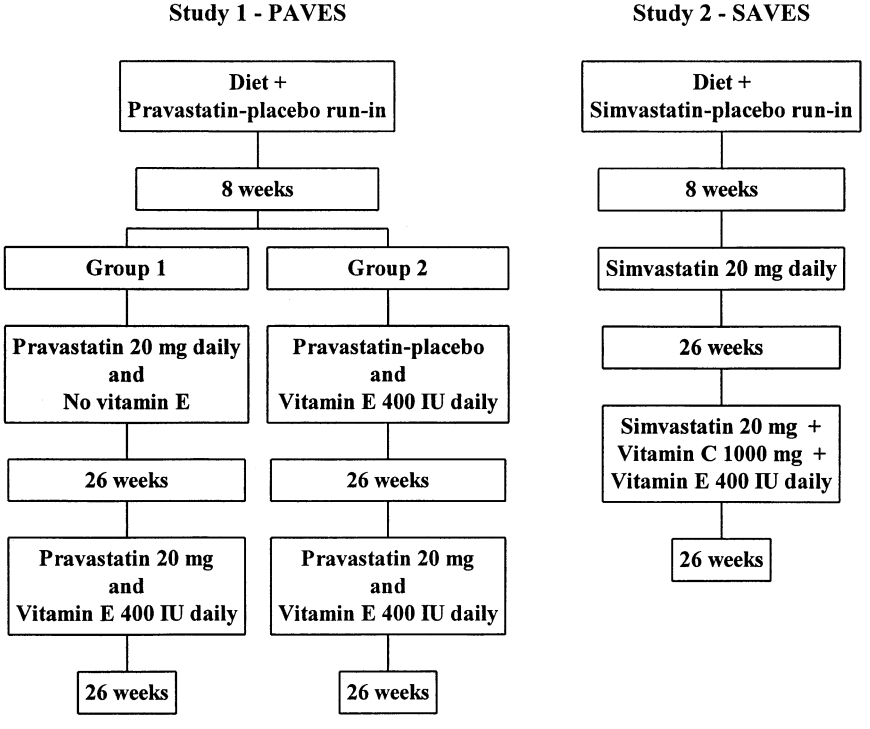

Figure 1. Study designs. PAVES = Pravastatin and Vitamin E in Seniors study; SAVES = Simvastatin, Ascorbate and Vitamin E in Seniors study.

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20 mg, vitamin C, 1,000 mg, and vitamin E, 400 IU (all d-alpha-tocopheryl), nightly for an additional 26 weeks.

Laboratory measurements. Subjects fasted for at least 12 h before laboratory tests. Lipid levels, liver function tests and creatine kinase levels were measured on a Hitachi 747 analyzer using standard reagents (Roche Diagnostics, Indianapolis, Indiana). Total serum cholesterol was measured by a cholesterol ester/oxidase enzymatic procedure. High-density lipoprotein cholesterol (HDL-C) was measured directly using enzymatic colorimetrics incorporating polyethylene glycol-modified cholesterol ester oxidase. Serum triglycerides were measured using a glycerol kinase-based enzymatic procedure. Low-density lipoprotein cholesterol was calculated by the Friedewald formula (29).

Brachial artery reactivity protocol. B-mode ultrasound was used to measure FMD of the brachial artery (30,31). Studies were performed at baseline and at subsequent visits on the morning of phlebotomy, after a 12-h fast. Subjects took their morning doses of prescribed medications. Brachial artery diameters and blood flows were measured with a 7.5-MHz linear array vascular ultrasound transducer and an Agilent Technologies 5500 Sonos ultrasound system (Andover, Massachusetts). Increased forearm blood flow was induced by inflating a blood pressure tourniquet around the widest part of the forearm to a systolic blood pressure of 250 mm Hg for 4.5 min. Repeat brachial artery diameter and blood flow scans were obtained immediately and 1 min after tourniquet deflation. Resting brachial artery diameter and blood flow scans were repeated 15 min later. Sublingual nitroglycerin (NTG) (400 μg) was administered, and final scans were performed after 3 min.

The brachial artery was imaged in longitudinal section 2 cm to 15 cm above the elbow. Images were recorded using the ultrasound digital storage and retrieval software system. Vessel diameters were measured in triplicate using digital calipers (MedArchive Viewer 2.1, Secure Archive, Boulder, Colorado). Measurements were performed blinded to subject information and treatment group. The brachial artery diameter was measured at end-diastole, using intima-media interfaces or, if not well-visualized, media-adventitia interfaces as landmarks. Flow-mediated vasodilation was calculated as the ratio of the brachial artery diameter after reactive hyperemia to the baseline diameter, expressed as a percent change. Nitroglycerin-mediated vasodilation (NTGMD) was calculated in an analogous fashion. Volumetric flow rates were calculated by multiplying the time velocity integral of the angle-corrected Doppler flow signal by the heart rate and the mean cross-sectional vessel area. Changes in blood flow were expressed as percentages of resting flow. In our laboratory, intraobserver reliability for measurement of the brachial artery diameter is 0.987, reflecting an interclass correlation coefficient across all readings and conditions (30). Because of poor image quality, data from two subjects in PAVES were excluded.

Statistical analysis. Clinical parameters included age, gender, lipid values, heart rate and blood pressure. Brachial artery reactivity parameters included brachial artery diameter, FMD and NTGMD. Clinical and brachial artery reactivity parameters were described by means and SD. Outcome parameters included changes in lipid values, brachial artery diameter, FMD and NTGMD. Changes within groups were assessed by paired *t* tests. In PAVES, between-group comparisons used *t* tests and chi-square tests. Between studies comparisons used analysis of variance.

Changes in FMD, the primary end point, were evaluated using the general linear mixed model for repeated measures, controlling for changes in baseline diameter (SAS System for Mixed Models, SAS Institute, Cary, North Carolina). Bonferroni's adjustment for multiple comparisons was performed with a repeated measures contrast procedure. Pearson product-moment correlation coefficients described linear relationships between parameters. Predictors of FMD were determined by linear forward step-wise regression analyses. Based on published sample-size nomograms and data from our laboratory, the number of subjects who completed each study provided 90% power to detect a 2.5% change in FMD within groups and 80% power to detect a 4% change in FMD between groups with an alpha of 0.05 (30,31).

RESULTS

Subject characteristics. **STUDY 1—PAVES.** Fifty-six subjects participated in the placebo run-in period, after which eight no longer met lipid criteria for randomization; three used prohibited medications; one withdrew because of a rash, and two withdrew for personal reasons. Of the 42 randomized subjects, three subjects in group 1 and two subjects in group 2 withdrew within one month of randomization because of side effects or for personal reasons. Accordingly, 37 subjects were included in the final data analysis. The average age in PAVES was 76.1 ± 4.3 years (range 71 to 86 years) (Table 1). Hypertension was present in 83% of the subjects, and the average systolic blood pressure was 145.4 ± 18.9 mm Hg. The most commonly used antihypertensive medications were beta-adrenergic blocking agents (22%), calcium-channel blockers (19%), diuretics (14%) and angiotensin-converting enzyme (ACE) inhibitors (11%). Three subjects (8%) were using estrogen replacement therapy. Use of these medications was similar in each group, with the exception of ACE inhibitors, which were more commonly used in group 1; however, two additional subjects in group 2 started ACE inhibitors during the study. Five subjects started diuretics, and three started beta-blockers. Dosages of vasoactive medications did not change significantly during the study. No subjects started estrogen replacement therapy. Subjects had elevated total cholesterol (267.7 ± 41.4 mg/dl) and LDL-C (185.7 ± 29.5 mg/dl) levels. Baseline characteristics and lipid levels did not differ between groups (Table 1).

STUDY 2—SAVES. Twenty-one patients participated in the placebo run-in period, after which one no longer met lipid criteria for study continuation. Of the 20 subjects who

Table 1. Baseline Subject Characteristics

	PAVES Group 1	PAVES Group 2	P*	SAVES	P†
n	17	20	—	17	—
Age (yrs)	76.7 ± 4.6	75.7 ± 4.1	0.482	74.8 ± 3.8	0.446
Women (n)	10	14	0.716	9	0.221
CHD (n)	5	4	0.506	3	0.685
Hypertension (n)	11	15	0.495	12	0.738
Diabetes mellitus (n)	0	1	0.380	1	0.649
Systolic blood pressure (mm Hg)	143.3 ± 19.9	146.3 ± 18.6	0.654	147.8 ± 22.8	0.817
Total cholesterol (mg/dl)	274.0 ± 51.9	262.7 ± 31.1	0.423	248.7 ± 23.4	0.150
LDL cholesterol (mg/dl)	189.6 ± 36.8	182.6 ± 22.6	0.486	168.9 ± 21.6	0.100
HDL cholesterol (mg/dl)	48.9 ± 8.8	51.3 ± 12.3	0.519	53.8 ± 13.9	0.504
Triglycerides (mg/dl)	170.6 ± 96.2	144.4 ± 58.9	0.321	130.4 ± 48.2	0.251
Brachial artery diameter (mm)	4.5 ± 0.9	3.8 ± 0.8	0.037	4.3 ± 0.7	0.047
Flow-mediated vasodilation (%)	−0.1 ± 3.5	3.1 ± 4.2	0.027	3.2 ± 3.1	0.023
Nitroglycerin-mediated vasodilation (%)	11.0 ± 4.7	10.3 ± 7.1	0.735	10.8 ± 4.8	0.930

*PAVES group 1 vs. group 2; †PAVES (all subjects) vs. SAVES.

CHD = coronary heart disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PAVES = Pravastatin and Vitamin E in Seniors study; SAVES = Simvastatin, Ascorbate and Vitamin E in Seniors study.

started therapy, three withdrew because of side effects or for personal reasons. Accordingly, 17 subjects were included in the final data analyses. The average age in SAVES was 74.8 ± 3.8 years (range: 70 to 82 years) (Table 1). Hypertension was present in 71% of the subjects, and the average systolic blood pressure was 147.8 ± 22.7 mm Hg. The most commonly used antihypertensive medications were diuretics (29%), beta-blockers (18%) and calcium-channel blockers (12%). Two subjects (12%) were using estrogen replacement therapy. Dosages of vasoactive medications did not change significantly during the study. No subjects started estrogen replacement therapy. One subject started using an ACE inhibitor; one started a calcium-channel blocker, and one started a beta-blocker. Subjects had elevated total cholesterol (248.7 ± 23.4 mg/dl) and LDL-C (168.9 ± 21.6 mg/dl) levels (Table 1). Baseline subject characteristics and lipid levels did not differ between studies (Table 1).

Changes in lipid levels. STUDY 1—PAVES. Vitamin E did not affect lipid levels (Table 2). In group 1, pravastatin reduced total cholesterol levels by 24%, LDL-C by 31%, triglycerides by 23% and raised HDL-C levels by 4% (all $p < 0.05$) (Table 2). The increase in HDL-C levels observed at 26 weeks no longer was statistically significant after 52 weeks. Similar results were seen in group 2. Total cholesterol (−22%) and LDL-C (−30%) levels decreased ($p < 0.001$), and HDL-C increased (+5%, $p < 0.05$) on pravastatin.

STUDY 2—SAVES. Simvastatin therapy for 26 weeks reduced total cholesterol levels by 24% ($p < 0.001$) and LDL-C levels by 36% ($p < 0.001$) (Table 2). High-density lipopro-

Table 2. Lipid Values

PAVES–Group 1	Baseline	26 Weeks (Pravastatin)	52 Weeks (Pravastatin + Vitamin E)
Total cholesterol	274.0 ± 51.9	207.7 ± 38.2*	209.9 ± 35.0*
LDL-C	189.6 ± 36.8	131.1 ± 29.7*	129.8 ± 25.2*
HDL-C	48.9 ± 8.8	50.5 ± 10.5‡	50.9 ± 10.8
Triglycerides	170.6 ± 96.2	130.6 ± 67.2†	142.5 ± 76.1‡
PAVES–Group 2	**Baseline**	**26 weeks (Vitamin E)**	**52 Weeks (Pravastatin + Vitamin E)**
Total cholesterol	262.7 ± 31.1	267.5 ± 42.5	209.0 ± 26.4*
LDL-C	182.6 ± 22.6	182.4 ± 36.6	128.4 ± 19.0*
HDL-C	51.3 ± 12.3	51.6 ± 13.1	54.2 ± 12.8‡
Triglycerides	144.4 ± 58.9	139.9 ± 55.5	126.5 ± 57.1
SAVES	**Baseline**	**26 Weeks (Simvastatin)**	**52 Weeks (Simvastatin + Vitamins C + E)**
Total cholesterol	248.7 ± 23.4	188.9 ± 32.7*	197.3 ± 16.6*
LDL-C	168.9 ± 21.6	107.8 ± 29.7*	117.5 ± 15.9*
HDL-C	53.8 ± 13.9	56.5 ± 14.1	54.2 ± 13.5
Triglycerides	130.4 ± 48.2	122.6 ± 54.5	123.8 ± 54.5

*$p < 0.001$ vs. baseline; †$p < 0.010$ vs. baseline; ‡$p < 0.050$ vs. baseline.

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PAVES = Pravastatin and Vitamin E in Seniors study; SAVES = Simvastatin, Ascorbate and Vitamin E in Seniors study.

Table 3. Brachial Artery Reactivity Data

PAVES—Group 1	Baseline	26 Weeks (Pravastatin)	52 Weeks (Pravastatin + Vitamin E)
Brachial artery diameter (mm)	4.5 ± 0.9	4.2 ± 0.9*	4.3 ± 0.9*
Resting flow (ml/min)	59.7 ± 35.5	57.9 ± 37.8	57.4 ± 25.1
FMD (%)	−0.1 ± 3.5	3.7 ± 3.8†‡	4.4 ± 4.0§
Reactive hyperemia flow (ml/min)	321.4 ± 156.0	298.9 ± 154.5	292.3 ± 133.1
NTGMD (%)	11.0 ± 4.7	10.7 ± 6.9	10.2 ± 5.7
Post-NTG flow (ml/min)	77.7 ± 30.7	70.4 ± 29.2	65.9 ± 25.5
PAVES—Group 2	**Baseline**	**26 Weeks (Vitamin E)**	**52 Weeks (Pravastatin + Vitamin E)**
Brachial artery diameter	3.8 ± 0.8	3.9 ± 0.8	3.8 ± 0.8
Resting flow	45.1 ± 23.7	50.4 ± 34.0	51.9 ± 26.0
FMD	3.1 ± 4.2	2.6 ± 4.1	5.3 ± 4.8
Reactive hyperemia flow	264.9 ± 113.5	227.4 ± 80.3	282.0 ± 128.6
NTGMD	10.3 ± 7.1	12.4 ± 5.7	10.7 ± 5.0
Post-NTG flow	72.6 ± 36.2	69.1 ± 47.3	57.3 ± 43.0
SAVES	**Baseline**	**26 Weeks (Simvastatin)**	**52 Weeks (Simvastatin + Vitamins C + E)**
Brachial artery diameter	4.3 ± 0.7	4.2 ± 0.7	4.4 ± 0.7
Resting flow	56.6 ± 41.7	49.4 ± 29.5	56.4 ± 18.6
FMD	3.2 ± 3.1	2.6 ± 3.2	2.9 ± 2.3
Reactive hyperemia flow	281.1 ± 135.4	270.9 ± 127.0	294.2 ± 129.2
NTGMD	10.8 ± 4.8	12.0 ± 6.4	8.9 ± 7.6
Post-NTG flow	57.2 ± 26.1	59.8 ± 26.9	50.2 ± 21.7

*$p < 0.010$ vs. baseline; †$p < 0.020$ vs. baseline; ‡$P_{adjusted} = 0.093$ vs. baseline; §$P_{adjusted} = 0.066$ vs. baseline.
FMD = Flow-mediated dilation; NTG = nitroglycerin; NTGMD = nitroglycerin-mediated vasodilation; PAVES = Pravastatin and Vitamin E in Seniors study; SAVES = Simvastatin, Ascorbate and Vitamin E in Seniors study.

tein cholesterol and triglyceride levels did not change significantly (Table 2). Adding vitamins C and E did not affect lipid levels. Reductions in total cholesterol and LDL-C remained statistically significant after 52 weeks.

Brachial artery reactivity. Resting and reactive hyperemia flow rates were similar and remained stable throughout both studies (Table 3). At baseline, FMD did not correlate significantly with total cholesterol, LDL-C, HDL-C or triglycerides. Flow-mediated vasodilation on treatment and changes in FMD did not correlate significantly with lipid levels or their changes.

STUDY 1—PAVES. At baseline, subjects in both groups had endothelial dysfunction characterized by impaired FMD of the brachial artery (Table 3). Because subjects in group 1 had larger baseline brachial artery diameters (4.5 ± 0.9 vs. 3.8 ± 0.8 mm, $p = 0.037$), FMD was lower in group 1 (−0.1 ± 3.5 vs. 3.1 ± 4.2%, $p = 0.027$, Table 1). Accordingly, between-group comparisons of FMD were adjusted for brachial artery diameter. In multivariate analysis, only brachial artery diameter and systolic blood pressure predicted FMD. Nitroglycerin-mediated vasodilation was similar in both groups (Table 3).

In group 1, the apparent increase in FMD ($p = 0.012$) after 26 weeks of pravastatin no longer was statistically significant after adjusting for multiple comparisons ($p = 0.093$, Table 3). Nitroglycerin-mediated vasodilation also did not change significantly ($p = 0.361$). Adding vitamin E to pravastatin did not affect FMD ($p = 0.586$) or NTGMD ($p = 0.510$) significantly (Table 3). In group 2, therapy with vitamin E for 26 weeks did not improve FMD ($p = 0.735$) or NTGMD ($p = 0.361$) significantly (Table 3). The small increase in FMD associated with the use of pravastatin was not statistically significant ($p = 0.097$), and NTGMD did not change ($p = 0.434$).

In comparisons between groups 1 and 2, the change in FMD with pravastatin was not different from the change in FMD with vitamin E, either at 26 weeks ($p = 0.444$) or at 52 weeks ($p = 0.773$).

STUDY 2—SAVES. At baseline, the average brachial artery diameter was 4.3 ± 0.7 mm, and subjects had impaired FMD of the brachial artery (3.2 ± 3.1%) (Table 3). After 26 weeks of therapy with simvastatin, FMD did not change significantly ($p = 0.677$) (Table 3). After 26 additional weeks of simvastatin and the addition of vitamins C and E, FMD still did not change ($p = 0.791$). Over the entire 52 week treatment period, FMD did not change as compared with baseline ($p = 0.958$) (Table 3). Nitroglycerin-mediated vasodilation did not change after 26 weeks of therapy with simvastatin ($p = 0.341$) or after 26 additional weeks of simvastatin with vitamins C and E ($p = 0.444$). Accordingly, after 52 weeks, NTGMD did not change from baseline ($p = 0.438$) (Table 3).

Combined analyses. All of the prepravastatin (baseline values from group 1 and 26 week values from group 2, $n = 35$) and postpravastatin (26-week values from group 1 and 52-week values from group 2, $n = 35$) values were pooled to

increase statistical power. In the pooled analysis from PAVES, FMD improved from 1.4 ± 4.0% to 4.6 ± 4.3%; however, after adjustment for repeated measures, this change was not statistically significant ($p = 0.051$). To further increase statistical power, all of the pre- and postsimvastatin (baseline and 26-week values from SAVES) were added to the pooled analysis from PAVES ($n = 52$). Flow-mediated vasodilation increased slightly from 2.2 ± 3.9% to 2.9 ± 3.7%; however, this change was not statistically significant ($p = 0.206$).

DISCUSSION

Effects of antioxidant vitamins. In these studies, treatment with vitamin E and the combination of vitamins C and E for up to 52 weeks did not improve FMD of the brachial artery in older adults with hypercholesterolemia, either alone or in combination with statins. Previous studies in middle-aged adults with hypercholesterolemia showed variable effects of vitamin E supplementation on endothelial function (20,21,23–28). Although oxidized breakdown products of vitamin E may be atherogenic when adequate levels of vitamin C are not present, the combination of vitamins C and E did not improve FMD in our studies. These findings are concordant with previous studies of this combination of antioxidant vitamins in younger patients with hypercholesterolemia (23) but are discordant with their acute effects after a fatty meal in healthy adults (22). Although use of vitamin C and E supplements was associated with a lower risk of total and coronary mortality in the Established Populations for Epidemiologic Studies of the Elderly (32), the results of the Heart Outcomes Prevention Evaluation and GISSI-Prevenzione trials suggest that vitamin E supplements do not reduce the incidence of future coronary events in patients with, or at risk for, CHD (33,34).

Effects of statins. In these studies, neither pravastatin nor simvastatin improved FMD of the brachial artery, either alone or in combination with antioxidant vitamins. Although short-term pravastatin therapy improved FMD in middle-aged adults with "average" and high cholesterol levels, similar lipid reductions did not improve FMD in our study of older adults (16,19). With one exception, subjects in our study had higher total cholesterol and LDL-C levels than previously investigated (16). In addition, our subjects were older than those in previous studies of lipid-lowering therapy and endothelial function. Our findings are concordant with a recent report that six months of simvastatin therapy did not improve coronary endothelial function in patients with CHD (35); however, they are discordant with another study in which simvastatin improved endothelial function of the forearm vasculature in patients with moderate hypercholesterolemia (18). That study, however, enrolled much younger subjects (mean age: 50 ± 2 years) and was only four weeks long. Although pravastatin and simvastatin did not improve FMD in our studies, they do reduce the incidence of adverse cardiovascular events in older adults (36–38). Thus, the beneficial effects of lipid-lowering therapy in older adults may be due to mechanisms other than improving FMD, such as plaque stabilization, reduction in inflammation or antithrombotic effects (39).

Effects of aging on vascular function. These studies are the first clinical investigations of the effects of vitamin C, vitamin E and lipid-lowering therapy on FMD in individuals ≥70 years old. Our subjects were older than those in previous investigations of these interventions on endothelial function.

Aging, per se, is associated with endothelial dysfunction (10–13). A 0.21%/year decline in FMD in men after age 40 years and a 0.49%/year decline in women after age 50 years has been reported (11). Aging-related structural changes in the vasculature, particularly in large- and medium-sized arteries, may contribute to impaired vasodilation. With aging, vessels lose distensibility as medial laminae and elastin fibers are lost, and elastin is replaced with collagen (12,40–42). Aging is also associated with reduced synthesis and release of endothelium-derived nitric oxide, increased activity of vasoconstrictive prostanoids, decreased diffusion of nitric oxide to smooth muscle cells and increased degradation of nitric oxide by oxygen-derived free radicals (12).

In both of our studies, NTGMD was lower than it was in studies of younger subjects with and without hypercholesterolemia. Nitroglycerin-mediated vasodilation did not change with antioxidant vitamin or statin therapy. In some studies, aging was not associated with impaired endothelium-independent vasodilation (11–13); however, in one of these studies, a negative trend was noted between age and NTGMD (11). Other studies have demonstrated inverse relationships between age and endothelium-independent vasodilation in women but not in men (14,43). In total, these studies included only six subjects ≥70 years old, the oldest of which was 73 years old (11,13). Although NTGMD improved with aging in one study, it only included two subjects ≥70 years old (44). Impaired NTGMD suggests that aging-related structural changes may lead to limited vasodilator reserve and may provide a ceiling effect to the maximum ability of arteries to dilate irrespective of endothelial function.

Study limitations. The brachial artery ultrasound technique for evaluating FMD is very sensitive and reproducible. Although PAVES and SAVES were relatively small studies, their sample sizes were larger than many published studies addressing the effects of these interventions on FMD in young and middle-aged adults. Their conclusions are strengthened by a longer duration of treatment and because each subject was used as his own control. Sample size estimates were based on published nomograms for FMD interventions and were verified using data from our laboratory (30,31). A small improvement in FMD (<2.5%) with pravastatin therapy cannot be excluded; however, in PAVES the small increase in FMD observed with pravastatin was not significant after adjusting for multiple comparisons, was

not seen in subjects already receiving vitamin E and only approached significance in the pooled group analysis. Furthermore, an improvement in FMD was not seen with simvastatin or in the combined analysis of both studies.

The dose of vitamin E used in these studies was less than that used in some trials (range 300 to 1,600 IU) that evaluated endothelial function in younger subjects; however, in those studies, the dose of vitamin E was not associated with the changes in endothelial function (20–28). Higher doses of vitamin E or a different preparation may be needed to demonstrate a treatment effect in older adults; however, its combination with vitamin C had no effect in our subjects. Plasma vitamin C and E levels were not measured, but the dose and preparation of vitamin E we used significantly reduced the in vitro oxidative susceptibility of LDL particles isolated from older adults with CHD (63.9 ± 9.8 years), indicating that the vitamin E used in our studies is a potent antioxidant (data unpublished). Furthermore, we verified adherence by counting vitamin C and E capsules and pills at each study visit.

Because the majority of our subjects had hypertension, elevated systolic blood pressures may have masked an improvement in FMD related to the interventions by providing a ceiling to the maximum possible improvement in FMD able to be achieved. Finally, impaired vasodilator reserve in older hypercholesterolemic patients also may have provided a ceiling to the maximum effectiveness of our interventions. If so, this questions the validity of using the brachial artery ultrasound technique for assessing endothelial function in older adults.

Summary. Older adults with hypercholesterolemia have impaired FMD of the brachial artery that does not improve with lipid-lowering therapy, alone or in combination with antioxidant vitamins. Because aging is associated with morphologic and physiologic changes in the vascular wall, the beneficial effects of lipid-lowering and antioxidant vitamin therapies on flow-mediated arterial dilation previously observed in younger adults may not occur in older adults. Reduced NTG-mediated arterial dilation in older adults may reflect impaired vasodilator reserve.

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Reprint requests and correspondence: Dr. James H. Stein, Section of Cardiovascular Medicine, University of Wisconsin Medical School, H6/315 Clinical Science Center (3248), 600 Highland Avenue, Madison, Wisconsin 53792. E-mail: jhs@medicine.wisc.edu.

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